Center Reflections

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California State University Fullerton

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CMolS Welcomes Staff Scientist Dr. Steven Herron

Herron received his Ph.D. at UCI, where his structural studies on pectate lyases revealed new insights about their catalytic mechanism and a shared active site motif with several other enzymes having little overall sequence homology.

In July 2001, Dr. Steven Herron joined CMolS as its permanent Staff Scientist. Dr. Herron did his undergraduate work at Brigham Young University in Provo Utah, where he double majored in Biochemistry and Molecular Biology. After graduating from Brigham Young University he worked at the Scripps Research Institute as a research technician. Dr. Herron worked with Drs. J. Greg Sutcliffe and Dan Gerendasy on elucidating the function of a brain specific protein, neurogranin/RC3. Using a number of different biochemical and biophysical techniques, including circular dichroism and fluorescence spectroscopy, Dr. Herron was able to characterize the interactions between neurogranin/RC3 and calmodulin. In a series of papers the Sutcliffe group was able to show that neuromodulin/RC3 regulates the threshold between long-term memory and long-term depression.

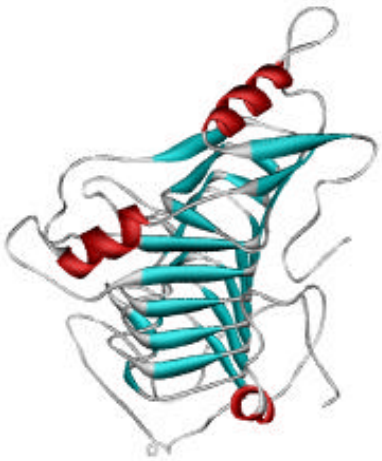
Dr. Herron did his graduate work, first at the University of California at Riverside, where he earned his Masters Degree in Biochemistry, and then at the University of California at Irvine, where he earned his Ph.D. At UCI, working in the laboratory of Dr. Francis Jurnak, he investigated the enzymatic mechanism of pectate lyases. Using a combination of crystallographic, biochemical, and kinetic methods, he was able to identify and elucidate the enzymatic mechanism of the pectate lyases.

Pectate and pectin lyases are pectolytic enzymes that degrade the middle lamella portion of the plant cell wall. These enzymes until recently were only found in plant pathogenic bacteria and fungal sources until the sequencing of the *Arabidopsis* genome. Gene annotation of the *Arabidopsis* genome identified over 25 putative pectate lyase genes, four of which have been confirmed. In plants, the role of the pectate and pectin lyases have yet to be determined, but inactivation of the putative pectate lyase protein pmr6 in *Arabidopsis* caused stunted growth and cupped leaves (J. Vogel, UCR, Personal communication).

In bacterial and fungal sources, the pectate and pectin lyases are extracellular proteins that degrade the plant cell wall causing tis-

sue damage and cell wall rupture. The most common sources of pectate lyases are the *Erwinia* bacterial species. The *Erwinia* spp. are relatively omnivorous pathogens, causing economic damage to a wide variety of plant crops including potatoes, carrots, radishes, onions, cucumbers, squash, eggplants, tomatoes, cabbages, celery, lettuce, and spinach. The infection can affect the plant leaves, stem, fruit, tuber, and roots. Affected plants become watery, turn black, and shrivel. Total decay of an entire tuber or fruit can occur within 3 to 5 days. The bacteria typically enter the plant through wounds produced by insects or by harvesting. The bacteria multiply in the intercellular spaces and secrete large amounts of pectolytic enzymes. Currently, proper insect control, avoidance of mechanical damage during harvesting, washing fruit or tubers with chlorinated water, and low temperature storage, are the only control methods available.

The pectate and pectin lyases have a unique fold, the parallel-beta-helix. Pectate lyase C was the first of the parallel beta-helix proteins to be characterized structurally (see figure below). However, because the pectate lyases had been only partially characterized previously, the enzymatic details and even the active site could not be determined once the structure was solved. To elucidate



the enzymatic mechanism, a number of inactivating mutants were created. Then, using plant cell wall fragments and a number of different inactivating mutants a substrate-mutant crystallographic complex was obtained. The substrate-mutant complex confirmed the postulated active site and identified a number of key amino acids involved in the binding of the substrate. Additional biochemical and kinetic experiments were required to further elucidate the enzymatic mechanism of pectate lyase C. From these experiments a clear model for the cleavage of pectate by pectate lyase C was determined.

While the work on the enzymatic mechanism of pectate lyase C was in progress, the structure of pectin lyase B was solved. Because the pectate and pectin lyases share a high degree of structural homology, structural superposition was possible. From the structural superposition of the substrate-mutant complex of pectate lyase C and native pectin lyase B, a clear, theoretically based substrate model could be built into the active pocket of pectin lyase B. Using Autodock, a theoretically based ligand docking program, a substrate-enzyme complex was modeled, which explained a number of differences between the pectate and pectin lyases.

Both the pectate and pectin lyases use a beta-elimination mechanism to cleave their substrate molecules. The beta-elimination reaction mechanism is a common reaction mechanism shared by a number of other enzymes, including the alginate lyases, the chondroitin lyases, enolase, mandelate racemase, and the muconate lactonizing enzyme. Using the available structural information a structural overlay of the active sites of several of these proteins was possible. While the enzymes had almost no sequence homology, an active site homology was clearly observed. The key amino acids in-

volved in the neutralization of the carboxylic acid group on the substrate and the proton abstraction step of the enzymatic reaction were spatially conserved in the active sites. Thus, a general spatially based requirement for the beta-elimination mechanism exists in proteins with little or no sequence or structural homology.

Since his arrival last July, Dr. Herron has been very busy, enthusiastically working on a number of projects at CMolS, both small molecule and macromolecular, which will be reported in future newsletters.

X-rays Make Smoother Chocolate

From: Elemental Discoveries
December 2001, Issue 48

For manufacturers of drugs and chocolate bars, an understanding of how they crystallize can mean the difference between a best-selling product and a flop. X-ray diffraction could help them get a clearer picture at the atomic level.

The taste and feel of chocolate in the mouth depends a lot on the crystal form of the cocoa solids, while some medicines work more effectively in one polymorphic form than another. Until now a crystal clear understanding at the atomic level of how different polymorphs form in everything from chocolate to medicine has been little more than trial and error except in the laboratory setting of the vacuum. Now, Elias Vlieg of the Department of Solid State Chemistry, at the University of Nijmegen, describes how X-ray diffraction (XRD) techniques can be used to study crystals as they form and so provide clues as to how their growth can be better controlled. The chance of tastier chocolate and more efficacious drugs is on the horizon.

If the growth of crystals were clear-cut, there would be no need to study crystal growth, but many compounds can crystallize in different - polymorphic - forms. Even a material as seemingly simple as carbon has several polymorphs - graphite, diamond and fullerite. The differences between polymorphs of the same compound can be tiny, an atom shifted slightly to the left, or a tighter angle between two bonds. But, they can also be quite large differences that impact on the overall properties of the solid. For a drug in solid form this can have a real impact on how well it is absorbed by the body. One polymorph may take longer to be dissolved and absorbed while another might be faster acting. The result can also alter the drug's side-effects. A slowly absorbed drug might sit in the stomach too long and cause irritation of the lining of the stomach for instance.

On the lighter side, the minute crystals of cocoa solids in a chocolate bar affect how the bar melts in the mouth. One crystal form may have a more pleasing texture on the tongue than another. According to Vlieg, XRD has been wholly successful in observing crystal growth in a vacuum. But for crystal growth from the more industrially realistic setting of a solution, melt or solid, it has until recently been little more than a dream tool.

Now, XRD is beginning to offer information on the structure of both sides of a growing interface. This, explains Vlieg, means that

structural details like relaxation and reconstruction on the crystal surface and ordering in the solution can be included in the theoretical description of crystal growth.

Understanding crystal growth in vacuum and beyond, Surface Science, In Press.

Crystallographic Studies of Diphosphinated Fischer Cr(0) and W(0) Carbenes

Cal Poly Pomona

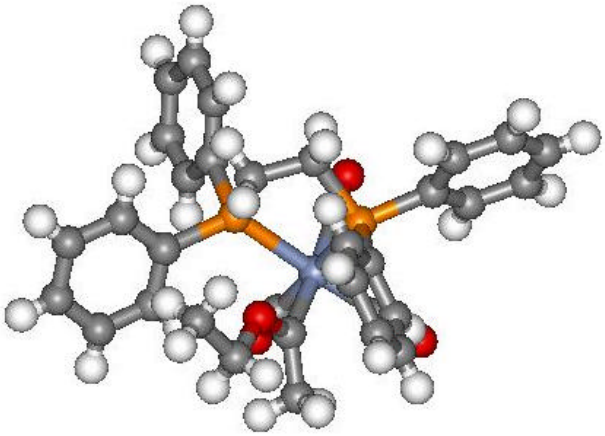
For the last few years, Joe Casalnuovo's research group at Cal Poly, Pomona, has been investigating the synthesis and reactivity of diphosphinated Fischer carbenes. Both alkoxy and amino Fischer carbene complexes have been synthesized, which have the general formulations $(CO)_3$(diphos)MC(OR)(R') and $(CO)_3$(diphos)MC(NR_2)(R'), respectively, including diphosphine (diphos) ligands such as DPPE, DPPP, and DPPM as well as chromium and tungsten metal centers (M). An important class of organometallic compounds, Fischer carbenes are synthetic intermediates in the production of a wide variety of organic functional groups, and notably used in the synthesis of natural products and amino acids. While non-phosphinated and monophosphinated Fischer carbenes have been known and characterized by X-ray crystallography since the 1960's, diphosphinated Fischer carbenes have been reported only very recently, and no X-ray structure determinations have been published.

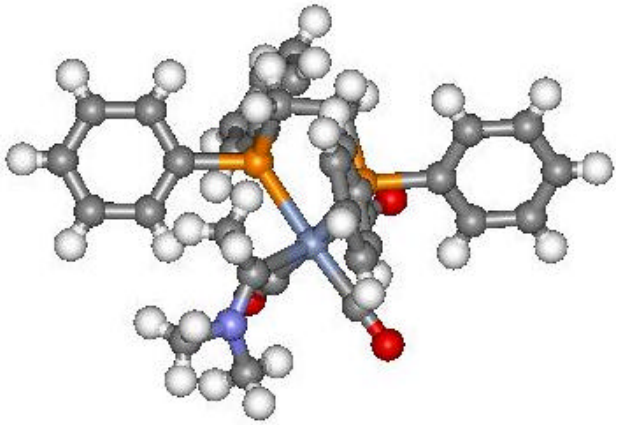
The interest in single-crystal X-ray determinations stemmed in part from a desire to see how the presence of the diphosphine ligand affected the structures of these novel compounds compared to the published structures of non-phosphinated or mono-phosphinated analogs. The series of diphosphinated compounds also makes for a nice structural study on their own since we can systematically vary the identity of the diphosphine ligand, the metal center, and the identity of the substituents on the carbene carbon.

In addition, while characterization of the complexes in solution provided good evidence for the coordination geometries adopted by these compounds, information about the specific orientation of the carbene moiety was more difficult to obtain. For example, $^{31}P\{^1H\}$ and $^{13}C\{^1H\}$ solution NMR studies indicated that the predominant product formed in each case had a *fac* type pseudo-octahedral geometry, although in most cases this species was in equilibrium with the *mer* isomer. Solution ^1H NMR spectra, however, while confirming the compositions of the compounds, suggested some differences between the spatial orientations of the carbene moieties in alkoxy-substituted Fischer carbenes versus the amino-substituted Fischer carbenes. Casalnuovo and his graduate student, Eric Reinheimer, turned to single-crystal X-ray diffraction to further explore these differences.

The diphosphinated Fischer carbenes synthesized in Casalnuovo's laboratory are readily crystallized. So far, Eric has worked with Dr. Katherine Kantardjieff at CMOLS to perform single-crystal X-ray analyses on nine different diphosphinated Fischer carbenes. Shown on the next page are renderings of two of the diphosphinated Fischer carbenes. The structure on the top is the alkoxy carbene, *fac*-$(CO)_3$(DPPE)CrC(OEt)(Me) while the structure on the bottom is the amino carbene, *fac*-$(CO)_3$(DPPE)CrC(NMe_2)(Me).

The views have been chosen to emphasize the orientation of the two carbene substituents relative to the two diphosphine phenyl rings that project toward the carbene moiety. In the case of the alkoxy carbene, it is the alkoxy substituent that is "sandwiched" be-





tween the phenyl rings while the alkyl substituent is directed away. By contrast, in the case of the amino carbene, it is the alkyl substituent that is "sandwiched" between the phenyl rings while the amino substituent is directed away. Indeed, these two examples illustrate a trend that carries through each of the eight structure determinations, four alkoxy carbene complexes and four amino carbene complexes.

In light of these results, the solution NMR spectra show that the solid state structural differences between diphosphinated alkoxy and amino carbenes are maintained in solution as well. Casalnuovo's laboratory is currently conducting studies comparing the reactivity of these respective carbene moieties; we are excited at the prospect of being able relate differences in their reactivity to the opposite orientations of their carbene substituents, as elucidated by the solid state X-ray analyses.

Eric Reinheimer received his B.S. degree in chemistry from Cal Poly, Pomona in June of 2000. He is currently working on completing his Masters degree at Cal Poly, Pomona where he also a Teaching Assistant in the freshman chemistry program. For the last year, Eric has divided his time between Cal Poly and working with Dr. Kantardjieff at CMolS to learn the science of X-ray crystallography. Eric was recently accepted in the Ph.D. program at Texas A&M University, where he will continue to focus on the use of X-ray crystallography in research in the laboratories of Drs. F. Albert Cotton and Kim R. Dunbar.

Joe Casalnuovo received his Ph.D. in inorganic chemistry from the University of Minnesota in 1990. He is an Associate Professor of Chemistry at California State Polytechnic University, Pomona where he focuses on teaching inorganic chemistry and supervising the freshman chemistry program. His research in the area of Fischer carbenes is carried out by a number of talented undergraduate and graduate students.

Websites of Interest

American Crystallographic Association
http://nexus.hwi.buffalo.edu/aca/index.html

International Union of Crystallography
http://www.iucr.org/

WinGX MS-Windows system of programs for solving, refining and analyzing single crystal X-ray diffraction data for small molecules.
http://www.chem.gla.ac.uk/~louis/software/wingx/

CRYSTOOL high efficiency screening protocol for crystals. http://www-structure.llnl.gov/crystool/crystool.htm

Autodock is a suite of automated docking tools. It is designed to predict how small molecules, such as substrates or drug candidates, bind to a receptor of known 3D structure.
http://www.scripps.edu/pub/olson-web/doc/autodock/

Cambridge Structural Database April 2002 release will contain 250,000th structure – REFCODE = IBEZAQ http://www.cdcc.cam.ac.uk

Upcoming Events

January 10-12, 2002: **CSUPERB Symposium**, Kellogg West Conference Center, Cal Poly Pomona. http://www.csuchico.edu/csuperb.

February 23-27, 2002: Biophysical Society Annual Meeting, San Francisco, CA. http://www.biophysics.org/annmtg/

February 25, 2002: **"Defining the Mandate of Proteomics in the Post-Genome Era"**, National Academy of Sciences Building, Washington D.C.

March 23 - 28, 2002: 9th International Conference on the Crystallization of Biological Macromolecules (ICCBM-9), Jena, Germany
http://www.conventus.de/iccbm9/

March 17-22, 2002: **PITTCON** New Orleans, LA. http://www.appcluster05.com/pittcon2002splash.cfm

April 7 - 11, 2001: **American Chemical Society** National Meeting, Orlando, FL. http://www.acs/org/meetings/

April 20-24, 2002: **Experimental Biology**, New Orleans, LA. http://www.faseb.org/meetings/eb2002/

April 21-26, 2002: **RapiData 2002 A practical course in macromolecular X-ray diffraction measurement.** Biology Department and National Synchrotron Light Source, Brookhaven National Laboratory http://px.nsls.bnl.gov/RapiData2002/

May 25-30, 2002: **American Crystallographic Association** National Meeting, San Antonio, TX. http://www.hwi.buffalo.edu/ACA/index.html

June 7-12, 2002: **8th Euro School on Electron Crystallography**, Tampere, Finland. http://conferences.tut.fi/ecschool2002

August 6-15, 2002: **International Union of Crystallography** Congress, Geneva, Switzerland. http://www.iucr.org/

November 10-14, 2002: **AAPS Pharmaceutica**, Toronto, Ontario, CAN. http://www.aapspharmaceutica.com/meetings/annualmeet/index.htm

Dear Friends and Fellow Crystallographers,

We are deeply shocked by the terrorists' attack against America. We scientists are by Nature international and cannot understand or accept such a horrible tragedy.

We shall even more than before, continue collaborating together to promote our science in all countries, especially the underdeveloped ones. We strongly believe and are convinced that this is a very important contribution to the world's peace.

With all our friendship,
Claude Lecomte, President, European Crystallographic Association

(from ACA Newsletter Winter 2001)

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